Case Report

Postmortem Distribution of Nicotine and Cotinine from a Case Involving the Simultaneous Administration of Multiple Nicotine Transdermal Systems*

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Abstract

A 31-year-old female was found dead with 18 nicotine transdermal system patches taped to her upper body and a plastic bag taped over her nose and mouth (the cause of death was ruled asphyxiation). Nicotine concentrations in biological fluids and tissues were analyzed using a liquid–liquid extraction followed by injection onto an HP-5890 gas chromatograph (GC) equipped with a nitrogen-phosphorus detector. Cotinine was separated from the biological matrices using solid-phase extraction followed by analysis on an HP-5890 GC with flame ionization detection. A variety of specimens were analyzed, including blood, urine, vitreous, brain, liver, and gastric contents. Heart and femoral blood concentrations (1.4 and 0.46 µg/mL, respectively) were 175 and 57 times, respectively, the mean C_{max} value reported following the proper administration of a single 7-mg/day patch.

Introduction

Nicotine has generated considerable medical interest because of its toxicity, its presence in tobacco products, and its propensity for producing dependence. It is a colorless, natural tertiary amine that has the odor of tobacco when exposed to air. Nicotine binds to nicotinic-acetylcholine receptors producing a variety of stimulant and depressant effects. The effects on the cardiovascular system include increased heart rate and vasoconstriction with the potential for vasospasm and cardiac arrhythmias. Lower doses act on the respiratory center of the medulla oblongata stimulating respiration. Higher doses depress respiration, which may result in respiratory failure and death in overdose.

Transdermal nicotine systems are used as substitution therapy for the cessation of smoking, and they provide a means of delivering nicotine to the systemic circulation via a route other than inhalation. There are currently four systems approved by the Food and Drug Administration (FDA): Habitrol® (CibaGeigy), Prostep® (Lederle), Nicotrol® (Warner-Lambert), and Nicoderm® (Marion Merrell Dow) (1). All of these systems are unique with different nicotine content and method of release into the circulation. The Nicoderm system, which was found in this case, is available in doses designed to deliver 7, 14, and 21 mg of nicotine over a 24-h period, which is the amount of time each patch is to be worn.

Absorption of nicotine through the transdermal system is gradual, with no rapid uptake of nicotine into the brain as occurs with the inhalational route of administration. Nicotine undergoes extensive biotransformation, primarily in the liver. Initially, the nicotine is oxidized to cotinine, which undergoes further metabolism to norcotinine and hydroxycotinine. Peak-plasma concentrations are achieved in 2–4 h, followed by a gradual decline over a 24-h period. At the end of the 24-h period, 68% of the nicotine released from the patch is absorbed, and the remaining 32% is believed to be lost through evaporation around the patch. A small quantity of nicotine is not released from the patch.

The development of transdermal drug delivery systems poses questions of pharmacokinetics and detectable drug concentrations for the toxicologist. What manner of toxicity can develop from the application of multiple patches? What drug concentrations will be attained from multiple, simultaneously administered systems? The authors are not aware of any articles reporting the simultaneous administration of multiple transdermal systems for the intent of committing suicide. This report describes what appears to be a such a case from the Office of the Chief Medical Examiner for the State of Oklahoma (OCME).

Case History

A 31-year-old female was found dead by her husband. She had a plastic bag taped over her nose and mouth. There was a second plastic bag containing vomitus near the body. Also near the body was a suicide note and an empty bottle of

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propoxyphene prescribed for her husband. Eighteen Nicoderm patches (7-mg dose) were attached to her chest and abdomen with duct tape. She was 53 inches tall, weighed 136 pounds, and was hydrocephalic.

Gross autopsy findings were essentially negative regarding cause of death. All toxicology testing was performed at OCME. Broad spectrum screening of the decedent's blood using procedures for gas chromatography (GC), gas chromatography–mass spectrometry (GC–MS), and radioimmunoassay was performed. Only nicotine, cotinine, and caffeine were detected. The cause of death was ruled asphyxia, and the manner of death was ruled suicide. The toxicology laboratory pursued the large concentrations of nicotine and cotinine found in the decedent's biological samples.

Materials and Methods

Reagents

Chem Elut columns (10 mL) were obtained from Varian Scientific (Harbor City, CA). Nicotine was obtained from Sigma Chemical (St. Louis, MO). Cotinine hydrochloride and glutethimide were purchased from Alltech (Deerfield, IL). Chlorobutane (Omnisolv®) was obtained from E.M. Science (Gibbstown, NJ). All other organic solvents were reagent grade and were acquired from Fisher Scientific (Plano, TX). All stock standards of nicotine and cotinine were prepared with methanol and stored at room temperature in amber bottles. No other special storage procedures were employed.

Preparation of the tissue samples involved the addition of 30 mL deionized water to 10 g of tissue in an 8-oz, plastic Oster blender cup (Thomas Scientific, Swedesboro, NJ). The cups were placed on an Oster Pulse-Matic blender and homogenized for approximately 1 min.

Nicotine extraction and analysis

The extraction procedure used for separating the nicotine from biologic matrices is an adaptation of the procedure published by Foerster et al. (2). Briefly, 0.5 mL concentrated ammonium hydroxide (NH_4OH) and 5 µg internal standard (*n*-propylamphetamine; NPA) were added to 2.0 mL of blood or 1 g of a 1:4 tissue homogenate in a clean 16 × 100-mm glass, screw-capped test tube. *N*-Butyl chloride (7.5 mL) was added, and the tubes were roto-extracted for 10 min with a model 151 multi-purpose rotator (Scientific Industries, Bohemia, NY). Following centrifugation for 5 min at 2000 rpms, the upper organic layer was transferred to a clean 16 × 100-mm tube. Sulfuric acid (1N, 2.5 mL) was added. The tubes were roto-extracted for 10 min and centrifuged for 5 min at 2000 rpm. The upper layer was aspirated to waste, and 0.5 mL NH_4OH and 2.5 mL *n*-butyl chloride were added. The tubes were again rotated for 10 min and centrifuged for 5 min. The upper layer was transferred to a clean, glass, 5-mL conical screw-capped tube and evaporated to dryness. The residues were reconstituted with 50 µL of methanol, and 1 µL was injected onto the GC.

The extracts were injected onto an Hewlett-Packard (HP) (Wilmington, DE) 5890 GC equipped with a fused-silica capillary column (Rtx 50, 15 m × 0.25 mm, 0.25-µm film thickness, Restek Bellefonte, PA) and a nitrogen-phosphorus detector. The injector and detector temperatures were 250 and 330°C, respectively. The analysis was accomplished using the following temperature program: 80 to 140°C at 10°C/min, then 140 to 300°C at 40°C/min. The samples were injected in the split mode at a ratio of 1:10.

Blood standards were prepared by spiking blank blood with concentrations of nicotine ranging from 0.25 to 4.0 µg/mL. The procedure was found to be linear across this range of concentrations, and the limit of detection was determined to be 0.1 µg/mL. Tissue concentrations were determined from the blood standard curves.

Extraction and analysis of cotinine

The extraction of cotinine was performed using a modification of the method reported by Anderson and Fuller (3). Blood (1 mL) or tissue (1 g of a 1:4 homogenate) was put into a 16 × 125-mm glass, screw-capped test tube along with 20 µg of glutethimide (internal standard) and 5 mL of 0.5M phosphate buffer (pH 5.5). The mixture was vortex mixed and poured into a 10-mL capacity ChemElut solid-phase extraction column (#1010, Analytichem International). The columns were allowed to stand after the last portion of sample had passed the frit. Two 12-mL volumes of methylene chloride were poured onto the column, waiting 3 min after the first 12-mL portion had passed the frit. Both eluents were collected, evaporated with nitrogen down to 3–5 mL, and transferred to a 5-mL glass, conical, screw-capped tube. The extracts were dried to residue and reconstituted with 200 µL of acetonitrile. The extracts were partitioned three times with 1-mL volumes of hexane, aspirating the hexane to waste each time. The acetonitrile layer was then evaporated to dryness, and the residues were reconstituted with 50 µL methanol.

The extracts (3 µL) were injected onto an HP-5890 GC equipped with a fused-silica capillary column (HP-I; 12 m × 0.20 mm, 0.25-µm i.d., Restek) and a flame ionization detector. Data collection was accomplished with an HP 3392A integrator. Temperature programming consisted of ramping from 100 to 220°C at a rate of 20°C/min. The injector temperature was 250°C, and the detector was set at 330°C.

Standard curves were constructed using blank blood that was spiked with various concentrations of cotinine and extracted as described previously. Tissue concentrations were determined using the standard curves prepared from blood. The assay was found to be linear over a range of 1 to 20 µg/mL. The limit of detection was found to be 0.1 µg/mL.

Results and Discussion

Nicotine toxicity is characterized by stimulation of the autonomic ganglia and central nervous system. As a result, a wide range of central and peripheral effects may be observed in overdose situations. Symptoms may range from abdominal pain, headache, and dizziness to convulsions, coma, and

respiratory arrest. Most cases of nicotine toxicity have resulted from oral ingestions of nicotine-containing solutions, such as pesticides (4), and accidental ingestions of used transdermal patches by children (5). Toxicity from the dermal absorption of nicotine has also been demonstrated in people who harvest tobacco (6).

Although the cause of death in the present case was ruled asphyxia because of the plastic bag taped over her nose and mouth, it was evident that the decedent was experiencing at least the gastrointestinal effects of nicotine overdose as demonstrated by the second plastic bag of vomitus found near the body. This is not surprising considering the dose of nicotine she would potentially receive from the 18 patches (7 mg) taped to her body (18 × 7 mg = 126 mg). This amount is roughly 2–3 times the estimated minimum lethal dose of nicotine in an adult (7). It is not known how long the patches were taped to her body, and the patches themselves were destroyed upon removal. Therefore, the exact dose that the decedent received was impossible to determine.

Table I shows the results of the analysis of various fluids and tissues from the decedent. Interestingly, the concentration of nicotine in the heart blood (1.4 µg/mL) was approximately three times that of the femoral blood (0.46 µg/mL). The phenomenon of postmortem redistribution is a possible explanation for this difference (8). Another explanation might be the location of all of the patches over the chest area, resulting in the absorption of the nicotine directly into the heart. Some of the higher concentrations of nicotine and cotinine were found in the liver and brain, which is not surprising because of the ease with which nicotine is able to cross cellular membranes and the blood-brain barrier. The last column reflects the ratio of nicotine to cotinine in each of the specimens analyzed. Interestingly, the ratios for the heart blood and brain are approximately 1.0 (1.07 and 0.89, respectively), whereas the ratios for liver and urine are less than 1.0 (0.38 and 0.52, respectively). It was expected that a greater proportion of metabolite would be found in the liver and urine, considering the role each of these plays in the metabolism and excretion of nicotine and cotinine. The greater ratio of nicotine to cotinine observed in the stomach contents (4:1) is an indication of the ionization, and thus "trapping", of nicotine in the stomach because of the acidic environment.

Table II reflects a comparison of plasma nicotine concentrations obtained after various routes of administration (9). The blood concentrations found in this case are well above those obtained with other routes, including the proper administration of a single 7-mg patch (10). In another report, transdermal patches, which were intended to deliver a dose of 22 mg of nicotine, that were administered to human subjects resulted in serum nicotine concentrations ranging from 4 to 444 ng/mL and serum cotinine concentrations of 35 to 249 ng/mL (11).

Table I. Nicotine and Cotinine Concentrations (µg/mL or µg/g)

Specimen	Nicotine	Cotinine	Nicotine–Cotinine
Heart blood	1.4	1.3	1.07
Femoral blood	0.46	–*	–
Urine	2.9	7.6	0.38
Vitreous	0.27	–	–
Brain	0.8	0.9	0.89
Liver	2.0	3.8	0.52
Gastric content	0.08	0.02	4.0

* Sample not tested.

Table II. Comparison of Plasma Nicotine Concentrations

Route	Concentration (ng/mL)	Reference
After pipe smoking	4.0–6.0	9
After cigarette smoking	18.3–22.0	9
After chewing 2 mg nicotine gum	11.8	9
After chewing 4 mg nicotine gum	23.2	9
1 Nicoderm patch, 7 mg/day, mean Cmax	8.0	10
Case: 18 Nicoderm patches (7 mg/day)		
Heart blood	1400.00	—
Femoral blood	460.00	

Conclusion

To our knowledge, this report is the first presentation of data from a case of attempted suicide with topical administration of multiple nicotine transdermal systems. The advent of this type of drug-delivery system has provided patients with a convenient method of drug administration and has been used for a number of therapeutic purposes, including the relief of pain, the prevention of motion sickness, and, as with this case, an aid for the cessation of smoking. This case has indicated the need for further study into the pharmacokinetics and potential toxicity from the administration of multiple transdermal systems.

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